

EVOLUTION

A morphological basis for path-dependent evolution of visual systems

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Path dependence influences macroevolutionary predictability by constraining potential outcomes after critical evolutionary junctions. Although it has been demonstrated in laboratory experiments, path dependence is difficult to demonstrate in natural systems because of a lack of independent replicates. Here, we show that two types of distributed visual systems recently evolved twice within chitons, demonstrating rapid and path-dependent evolution of a complex trait. The type of visual system that a chiton lineage can evolve is constrained by the number of openings for sensory nerves in its shell plates. Lineages with more openings evolve visual systems with thousands of eyespots, whereas those with fewer openings evolve visual systems with hundreds of shell eyes. These macroevolutionary outcomes shaped by path dependence are both deterministic and stochastic because possibilities are restricted yet not entirely predictable.

Establishing the extent to which the evolutionary trajectories of complex systems are contingent on historical events, a phenomenon called path dependence (1, 2), is fundamental for understanding the predictability of evolution. If there is a single, locally optimal solution to an environmental problem, then evolution will tend to be predictable; if many functionally similar solutions exist, then evolution will tend to be unpredictable (3–6). Path dependence occurs when evolutionary trajectories contain “critical junctions,” which we define as events that commit lineages to one of multiple possible evolutionary pathways, thereby constraining the suite of possible outcomes. Path dependence is well established for the evolution of particular proteins in unicellular laboratory systems [e.g., (7–13)]. Although specific evolutionary outcomes are restricted by earlier events in some natural systems [e.g., (14–16)], path dependence is very difficult to establish in any system outside of the laboratory.

Demonstrating path dependence in natural systems is challenging because it requires the identification of critical junctions and elucidation of the constraints that those junctions impose on future evolutionary paths (17–20). First, critical junctions are difficult to identify because alternative evolutionary pathways are often not observable along the singular history of life. Path dependence may be inferred from convergent origins of complex traits because these events illustrate multiple evolutionary pathways, analogous to replicates in controlled laboratory experiments. If splits in evolutionary trajectories lead to functionally similar outcomes in separate lineages, then those splits

could be identified as critical junctions. These functionally similar outcomes may still differ in form, so critical junctions may act to direct evolutionary pathways toward functionally similar but morphologically distinct outcomes. Second, even if convergent evolution reveals potential critical junctions, convergent evolution of traits occurs most commonly in organisms with very different body plans and ecologies, which are likely to exert different selective pressures on traits. Therefore, most instances of convergent evolution are not effective replicates for establishing path dependence (12). Finally, accurately reconstructing the evolutionary histories of convergent traits requires understanding of ancestral conditions and knowledge of the timing of key transitions in character states. This requires a detailed fossil record and/or a robust phylogenetic history beyond that which is available for many lineages. Together, these obstacles make identification of critical junctions and path dependence in natural systems enormously challenging.

By overcoming the challenges imposed by other natural traits, the visual systems of chitons (Mollusca; Polyplacophora) provide a compelling case to test hypotheses about path-dependent evolution. First, morphologically distinct visual systems may have evolved separately in different lineages of chitons (21). Chiton visual systems likely evolved from aesthetes, which are numerous, microscopic sensory organs embedded in the eight articulating shell plates of these heavily armored mollusks (22). Aesthetes likely have multiple sensory functions, including sensitivity to light, but they do not confer vision (21, 23, 24). In most chitons, nerves from aesthetes run through narrow channels in the shell plates before exiting through slits at the edges of each plate (Fig. 1, A and B) (25, 26). In some lineages, pigmented clusters of photoreceptors (20 to 35 μm wide), hereafter referred to as eyespots, are attached to

aesthetes (Fig. 1, D, G, and J) (27, 28). In other lineages, the aesthetes are interspersed with camera-type eyes with image-forming lenses made of shell material (up to 145 μm wide), hereafter referred to as shell eyes [(22, 29–32); see Fig. 1, C, F, I]. If eyespots and shell eyes evolved separately in chitons, then these distributed visual systems may represent distinct evolutionary paths to a convergent functional outcome: spatial vision. Indeed, computational modeling and behavioral experiments indicate that both the eyespot- and shell eye-based distributed visual systems of chitons provide spatial vision (26, 31–33). Second, chitons have a relatively rich fossil record, permitting time-calibrated phylogenetic analyses (34). If the distributed visual systems of chitons have recent origins, then their evolutionary histories may be reconstructed with greater confidence than those of other visual systems, which largely have ancient histories (35). Finally, fossil and extant chitons are found in similar environments and thus tend to be ecologically similar: Most species live (or lived) on hard substrates in intertidal or shallow subtidal habitats. The body plan present in both fossil and extant chitons is consistent across clades and evolutionary time (36–39). Species exhibiting the full range of shell-embedded sensory organs can even be found living on the same rock (40). Here, we investigated whether the evolution of distributed visual systems in chitons is path dependent by mapping the origins of eyespots and shell eyes onto the most comprehensive chiton phylogeny produced to date. We then used the rich fossil record of chitons to time-calibrate our phylogeny, and graphed outcomes onto a phylomorphospace to identify specific points where earlier events committed some lineages to one or another specific evolutionary pathway, resulting in multiple solutions to the evolution of spatial vision. Our discoveries show that the evolution of complex visual systems, which are often portrayed as deterministic [e.g., (41)], is path dependent: Events at specific points (critical junctions) constrain lineages to one of a subset of possible pathways.

Chitons rapidly evolved visual systems four times in two distinct forms

To characterize patterns of visual system evolution in chitons, we used genomic target capture and Bayesian inference to produce the most complete phylogeny of chitons to date, with emphasis on Chitonina, the suborder that includes more than half of all extant chiton species and most species with eyespots or shell eyes. We found that distributed visual systems evolved separately in chitons at least four times: two lineages through eyespots and two other lineages through shell eyes (Fig. 2, A and B). The two lineages that contain species with eyespots, Callochitonida (28) and Chitonidae: Chitoninae (27, 42), are distantly related to

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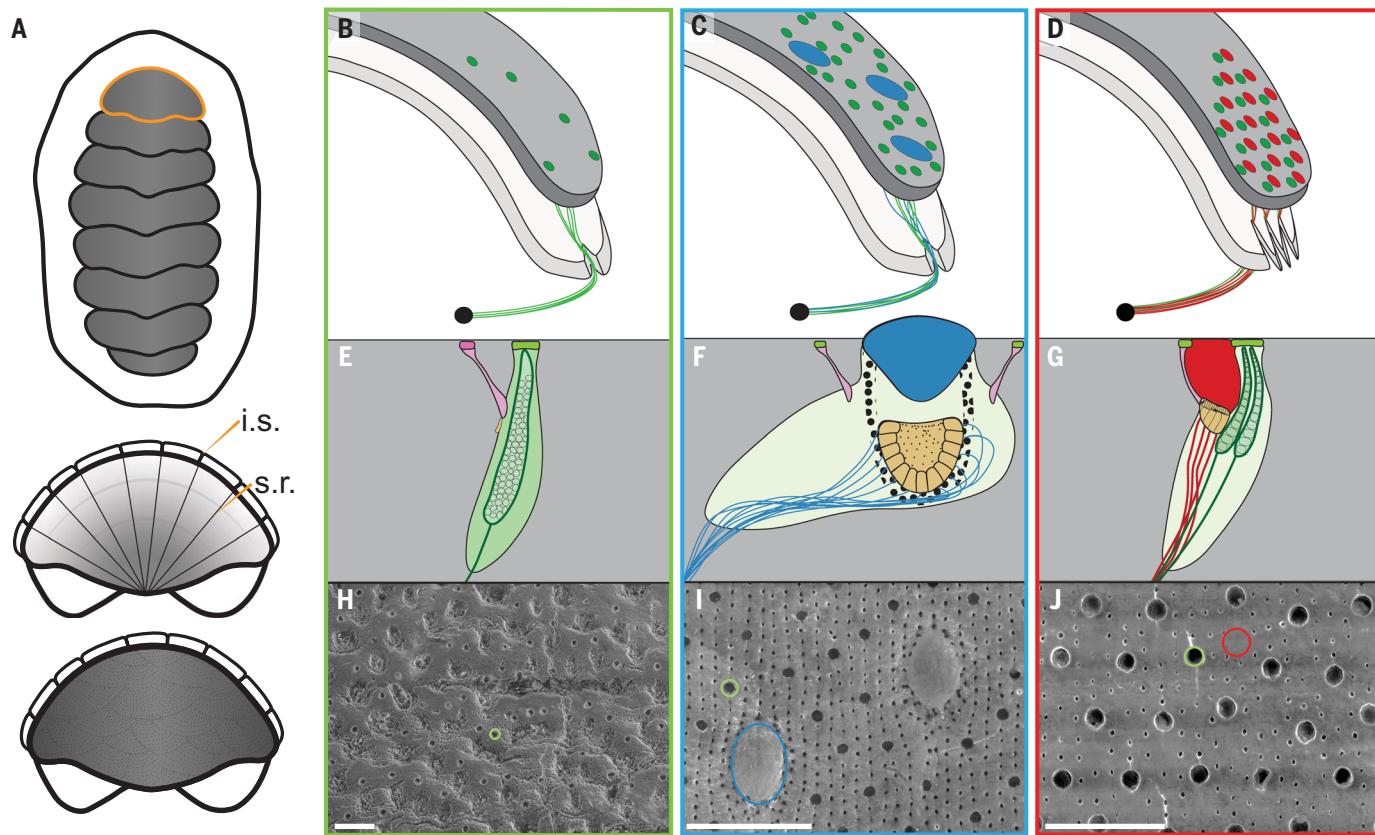


Fig. 1. Sensory organs embedded in chiton shell plates differ in morphology.

(A) A chiton (top) with the most anterior shell plate outlined in orange and popped out below. On the ventral side (middle), slit rays (s.r.) are visible leading to each insertion plate slit (i.s.). On the dorsal side (bottom), only insertion slits are visible. (B) Distribution of aesthetes (green) on the shell plate of a chiton with only aesthetes. Nerves (green lines) from aesthetes run through channels in the tegumentum, the visible outer layer of the shell (gray), exit the shell plate through an insertion slit, and then join the lateral neuropil (black circle), a part of the chiton nervous system. (C) Distribution of aesthetes (green) and shell eyes (blue) on the shell plate of a chiton with shell eyes. The density of aesthetes is higher, and nerves from both aesthetes and shell eyes travel through the shell plate to exit through an insertion slit. (D) Distribution of aesthetes (green) and eyespots (red) on the shell plate of a chiton with eyespots. Each eyespot is paired with one aesthete, and nerves from both aesthetes and eyespots travel through the shell plate to exit through several insertion slits. (E) Internal morphology of an aesthete, the simplest sensory structure embedded in the upper layer of the shell plate (gray). Here, a macraesthete (green) is depicted with a microaesthete (pink) branching

from it. (F) Internal morphology of a shell eye, with photoreceptor cells forming a retina (orange) beneath a lens (blue) and a large optic nerve running through the upper layer of the shell plate (gray). (G) Internal morphology of an aesthete with an attached eyespot, with a patch of photoreceptor cells (orange) beneath a clear portion of the shell plate (red) and a nerve running through the upper layer of the shell plate (gray). Note that panels (B) to (G) are not to scale. (H) SEM image of the surface of an anterior shell plate from *Katharina tunicata*, a chiton with aesthetes only. The location of a single macraesthete is circled in green. Scale bar, 100 μ m. (I) SEM of the surface of an anterior shell plate from *Acanthopleura brevispinosa*, a chiton with shell eyes. The location of a single macraesthete is circled in green, and a single shell eye is circled in blue. Scale bar, 100 μ m. (J) SEM image of the surface of an anterior shell plate from *Chiton marmoratus*, a chiton with eyespots. The location of a single macraesthete is circled in green, and a single eyespot is circled in red. Note that eyespots are connected to macraesthetes but appear as open regions of shell plate on SEM. The pigment of eyespots is only visible through decalcification of shell plates. Scale bar, 100 μ m.

one another, consistent with previous molecular phylogenies (43–45). Likewise, distributed visual systems based on shell eyes also evolved twice separately, once in Chitonidae: Acanthopleurinae + Toniciinae and once in Schizochitonidae, which in our phylogeny is a sister to the rest of Chitonina, making it a distant relative of Acanthopleurinae + Toniciinae. The placement of the sole extant genus of Schizochitonidae, *Schizochiton*, has been uncertain across studies of chitons (43, 46, 47) in part because *Schizochiton* contains only two accepted species, *S. incisus* (46) and *S. jousseaumei* (Dupuis, 1917), and *S. incisus* were the only speci-

mens available. Nevertheless, we are confident in our results because tests of branch stability do not indicate phylogenetic uncertainty in the placement of *S. incisus* in any of our analyses (see the supplementary materials, section 5.3, “Leaf instability testing”).

Next, to assess support for independent origins of distributed visual systems in chitons, we performed ancestral state reconstruction (ASR). Using ASR, we found high support ($\geq 95\%$ proportional marginal likelihood) for all four instances of visual system evolution in chitons occurring independently. Not only all Chitonina, but all living chitons and even ancient fossil poly-

placophorans (38) have or had aesthetes, but, as ASR reveals, eyespots and shell eyes are recent and nonhomologous additions to chiton sensory systems. Eyespots evolved independently in Callochitonida and Chitonidae: Chitoninae, and likewise shell eyes evolved independently within Chitonidae: Acanthopleurinae + Toniciinae and in Schizochitonidae. Within Chitonidae, eyespots evolved in a subclade in Chitoninae and shell eyes evolved in the last common ancestor of the other two subfamilies, Acanthopleurinae and Toniciinae. ASR showed that these visual systems evolved separately: The last common ancestor of Chitonidae only had aesthetes (95%

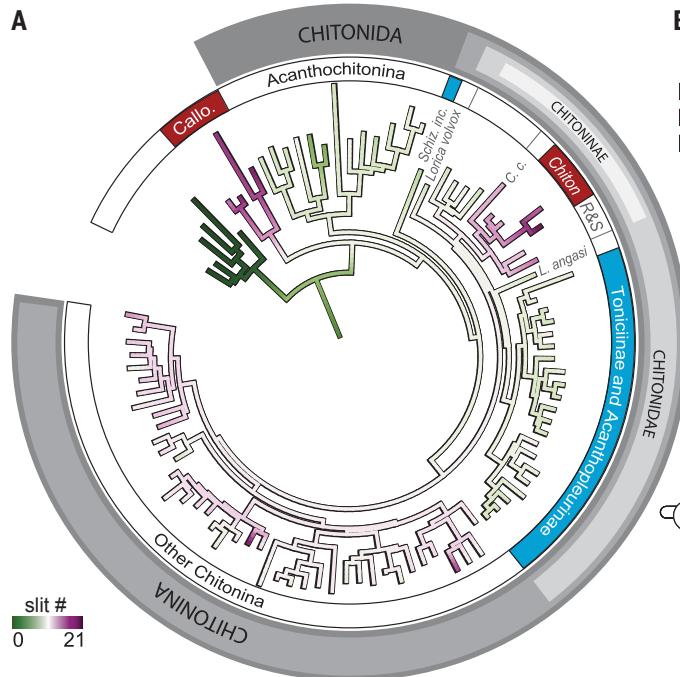
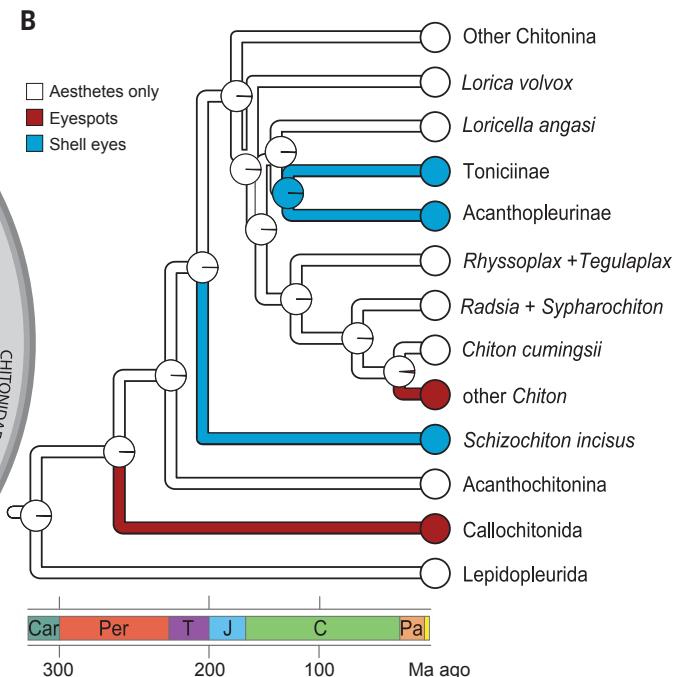


Fig. 2. Two types of distributed visual systems evolved convergently in chitons, one based on eyespots (red) and the other on shell eyes (blue). (A) The full maximum likelihood phylogeny of chitons produced by this study (outgroups not shown). Branch coloration indicates ASR of the number of slits in the anterior shell plate, where dark green represents 0 slits and pink represents >10 slits. Inner ring indicates the sensory organs embedded in the shell plates of taxa (aesthetes only, aesthetes and eyespots, or aesthetes and shell eyes), as well as phylogenetic affiliations.

proportional marginal likelihood). Thus, each of the four separate origins of visual systems in chitons is inferred to have evolved independently, including convergent origins of two different types of distributed visual systems, one based on eyespots and the other on shell eyes (Fig. 2A). Further, we found that eyespots and shell eyes evolved from aesthetes separately in Chitonidae, rather than eyes evolving from eyespots that evolved from aesthetes, a stepwise pattern that would have corresponded to the relative levels of morphological complexity demonstrated by these sensory organs.

To understand the timing of separate origins of visual systems in chitons, we used fossil occurrence data to time-calibrate the phylogeny and found that all four convergent visual systems in chitons evolved within the past 260 million years (Fig. 1B and fig. S8). Our time-calibrated phylogeny indicates shell eyes evolved in the last common ancestor of Acanthopleurinae + Toniciinae between 150 and 100 million years (Ma) ago and in Schizochitonidae between 250 and 200 Ma ago, estimates considerably older than the earliest verified fossil evidence of shell eyes in fossil Toniciinae from the middle Eocene (48 to 38 Ma ago) and fossil Schizochitonidae represented by two species of *Incisiochiton*



Outer ring indicates broader phylogenetic groups of chitons (gray boxes). (B) Time-calibrated phylogeny generated with Bayesian inference showing four independent origins of visual systems in chitons. Divergence times correspond to the geologic time scale below. ASR implies that all origins of eyespots or shell eyes in chitons come from an aesthete-only starting point, where all proportional marginal likelihoods are >95%. Additional support metrics are included in the supplemental materials (tables S2 and S8 and figs. S2, S3, and S9).

from the Lower Paleocene (66 to 59.2 Ma ago) (48–50). Time-calibrated phylogeny and fossil evidence together indicate that both instances of shell eyes in chitons represent the most recent origins of camera-type eyes known. By comparison, the more ancient camera-type eyes of vertebrates and cephalopods originated at least 500 and 425 Ma ago, respectively (35, 51). Distributed visual systems based on eyespots may have evolved even more recently than those based on shell eyes. Time-calibrated phylogeny and fossil evidence places the origin of eyespots in *Chiton* between 25 and 75 Ma ago. By contrast, eyespots in *Callochiton* could be as old as 260 Ma ago, but this date is less certain.

To determine how rapidly chitons evolved visual systems based on eyespots, we quantified the time between the origin of eyespots in *Chiton* and the most recent ancestor in Chitonina lacking pigmented eyespots. Using our fossil-calibrated time tree, we found that eyespots in *Chiton* originated within a period of seven million years. Theoretical models estimate that eyes can evolve within 363,992 generations (3, 52), so if we assume that chitons have a generation time of 3 years [based on available studies of other chiton genera, e.g., (53)], then a lineage of chitons could evolve a

visual system in 2 million years, within an order of magnitude of the 7 million years that we estimate for visual system evolution within *Chiton*. For comparison, the only published estimate of the time required to evolve an eye is from vertebrates, in which eyes evolved in ~30 million years (35, 54). Recent origins of shell eyes and eyespots in chitons allow us to calibrate the timing of visual system evolution with greater confidence from fossils. Thus, the recent origins and rapid evolution of visual systems in chitons make them particularly valuable for understanding how complex traits evolve.

Number of slits in shell plates is a critical junction in the evolution of chiton visual systems

To demonstrate path dependence in natural systems, it is first necessary to identify critical junctions. To discover critical junctions during the evolution of visual systems in chitons, we examined morphological differences between the shell plates of species with only aesthetes, species with eyespots, and species with shell eyes, and examined ancestral states for these traits. Most chitons integrate their shell-embedded sensory organs into their nervous system by passing nerves through slits along the edges of

their shell plates [(22, 25, 46, 55, 56); fig. S5]. In species with shell eyes, these slits are a vital part of the distributed visual system, because they make space for optic nerves to exit shell plates and make synaptic contact with the central nervous system (21). We predicted that as lineages of chitons added new types of sensory organs to their shell plates (like eyespots or shell eyes), they would require larger or more numerous slits for additional nerves to pass through. We compiled data on the number of slits in the anterior shell plates of all chitons in our phylogeny (table S2). Chitons may add slits to their shell plates as they grow, but slit number is not correlated with body size in chitons (larger chiton species do not have more slits) (fig. S14). Members of the clade sister to all remaining extant chitons, Lepidopleurida, lack slits. They innervate their aesthetes by running nerves through pores in their relatively thin shell plates, and outgroup comparisons indicate this is the ancestral state of crown-group chitons [table S2; (34, 57)]. In the remaining orders of chitons, Chitonida and Callochitonida, species have thicker shell plates, so slits are necessary to innervate aesthetes; all of these chitons have at least five slits on their anterior shell plates, and most have fewer than 10 (37). Like most chitons that only have aesthetes, most extant species with shell eyes have 10 or fewer slits on their anterior shell plates, including *S. incisus*, which has seven, and the inferred ancestor of Acanthopleurinae + Toniciinae (fig. S8), which has eight. In contrast to species with shell eyes, all extant chitons with eyespots that we examined have anterior shell plates with between 14 and 21 slits. We thus hypothesized that an increased number of slits is a critical junction in the evolution of visual systems in chitons, favoring the evolution of eyespots but not shell eyes.

If an increased number of slits is a critical junction that imposes a functional constraint favoring the evolution of eyespots instead of shell eyes, then an increase in the number of slits will predate the origin of eyespots themselves. Within Chitoninae (Fig. 2B), we compared the number of slits between species with eyespots and those with only aesthetes. All species with eyespots that we examined had ≥ 14 slits across their anterior shell plates. *Chiton cumingsii*, sister to the remaining members of *Chiton* in our phylogeny, does not have eyespots but has 14 slits (table S2 and personal observation by D.E. and D.I.S.). Sister to *Chiton*, species in the *Radsia* + *Sypharochiton* clade, all of which lack eyespots, have 13 to 16 slits. Sister to *Chiton* + *Radsia* + *Sypharochiton*, species in the *Rhyssoplax* + *Tegulaplast* clade have eight to 10 slits. We performed ancestral state reconstruction and found that the ancestors of Chitonidae and of Chitoninae each most likely had eight or nine slits, whereas the last common ancestor of *Chiton* + *Radsia* + *Sypharochiton*,

which we infer lacked eyespots, likely had 12 to 13 slits (fig. S8). Therefore, slits became more numerous in the *Chiton* + *Radsia* + *Sypharochiton* clade before the evolution of eyespots within *Chiton*. In Callochitonida, the other clade of chitons that evolved eyespots, an eyeless sister species or clade is unavailable because all Callochitonida examined to date appear to have eyespots (28). All species of Callochitonida in our analysis had > 16 slits. Ancestral state reconstruction suggests that the last common ancestor of Callochitonida

and Chitonida had eight or nine slits, indicating an independent origin of the increased number of slits in Callochitonida (fig. S8). In chitons, the evolution of eyespots appears to follow an increase in slit number, but the evolution of shell eyes does not, indicating that slit number is a critical junction during visual system evolution.

A phylomorphospace supports path-dependent evolution of chiton visual systems

In light of the four separate origins of visual systems in chitons and of the different morphological characters associated with them, we hypothesized that evolutionary outcomes in chiton visual systems were constrained by path-dependent evolution. From this hypothesis, we predicted that critical junctions have resulted in gaps in the phylomorphospace of chiton visual systems due to the absence of intermediate forms: Chitons can have either eyespots or shell eyes, but not visual systems that share morphological characters with both.

To test our hypotheses about path-dependent evolution in chitons, we constructed a phylomorphospace based on morphological traits associated with visual systems: aesthete density and number of slits in anterior shell plates. From museum specimens representing taxa across our phylogeny (fig. S4), we removed anterior shell plates and counted slits. We then quantified macraesthete densities (Fig. 1, H to J, green circles) from scanning electron microscope (SEM) images of these same plates and plotted these values alongside the number of slits in each species. Consistent with our prediction, we found a pronounced gap in the phylomorphospace, which suggests that slit number acts as a constraint on the type of visual system that a lineage of chitons can evolve (Fig. 3). The absence of intermediates in the phylomorphospace of chiton visual systems shows that chitons evolved vision through one of two distinct paths and suggests that the morphological characters that define each type of visual system are mutually exclusive. As in our ASR analysis, our phylomorphospace analysis indicated that increases in slit number preceded the evolution of eyespots in chitons. The number of slits in the anterior shell plates of chitons with eyespots was consistently higher than the ancestral number of slits in those lineages, and only those lineages of chitons that increased the number of slits in their plates evolved eyespots. Slit number therefore acts as a critical junction, an event that commits a lineage of chitons to one or the other evolutionary pathway toward spatial vision despite being unrelated to vision at the time of change. These critical junctions make the evolution of visual systems in chitons path dependent, and they are visible as a gap in phylomorphospace created by mutually exclusive solutions for spatial vision that are morphologically divergent yet functionally convergent.

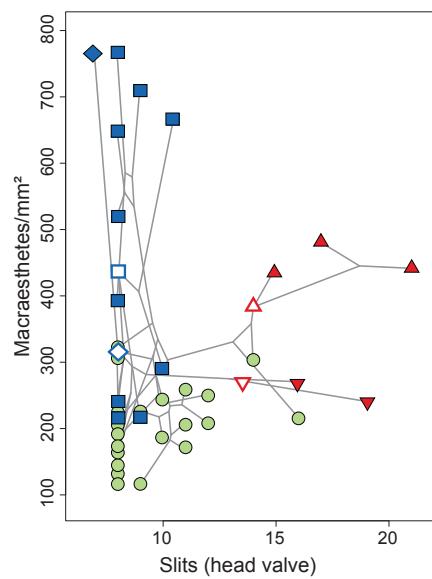


Fig. 3. Path dependence of visual system evolution in chitons is indicated by a phylomorphospace of vision-related morphological traits, showcasing two mutually exclusive solutions to vision. Phylomorphospace of chiton visual systems with number of slits on the x axis and aesthete density (in aesthetes/mm²) on the y axis (37 species). Lepidopleurida are excluded for visualization, but a complete phylomorphospace is available in fig. S12 (39 species). There are two separate origins of shell eyes (blue squares indicate members of Acanthopleurinae + Toniciinae, and the blue diamond indicates the position of *Schizochiton incisus*) and two separate origins of eyespots (red triangles indicate the members of Chitoninae with eyespots and red inverted triangles indicate the members of Callochitonidae). Green circles indicate chitons with only aesthetes. As predicted, there is a gap in the morphospace, indicating that slit number is a critical junction in chiton visual system evolution. The gap results from the absence of intermediate forms of visual systems in chitons. Lineages are committed to one or the other visual system at a critical junction (the central split in lineages). Ancestral states of each lineage of chitons with either shell eyes or eyespots are included as open polygons of shapes corresponding to those denoting extant species.

Increases in aesthete density are associated with the evolution of visual systems in chitons, but aesthete density is not a critical junction because it does not constrain the type of visual system that a lineage can evolve. Most chitons with either shell eyes or eyespots have a greater density of aesthetes than chitons with only aesthetes (Fig. 3). The ancestral states predicted for both clades of chitons with shell eyes (Fig. 3, open blue square and open blue diamond, and fig. S11) and the clade in Chitoninae with eyespots (Fig. 3, open red triangle, and fig. S11) indicate increases in aesthete density before or concurrent with the evolution of distributed visual systems. Because species in all four lineages that gained visual systems tend to have denser arrays of aesthetes than their sister lineages, an increase in aesthete density may be a preadaptation for a lineage of chitons evolving a visual system.

Slit number is a critical junction and not a preadaptation because slit number constrains the type of visual system that may evolve, but slit number can increase without the subsequent evolution of a visual system. When slit number increased in a lineage of chitons but aesthete density did not (Fig. 3, green circles), neither eyespots nor eyes evolved, emphasizing the role of increased aesthete density as a preadaptation. The evolution of visual system type in chitons is thus constrained, rather than being deterministic or stochastic: When aesthete density increases alongside slit number, lineages can evolve eyespots, and when aesthete density increases but slit number does not, lineages can evolve shell eyes.

Conclusions

Convergent evolution is often portrayed as an inevitable feature of lineages moving toward an optimal solution to an environmental problem (58). Such arguments dismiss contingency as a lesser force than selective pressure and assert that, given sufficient time, an optimized trait will evolve in a deterministic manner. However, chiton visual systems present a morphospace with multiple solutions: Networks of either eyespots or shell eyes provide chitons with spatial vision. We found evidence for a critical junction that defines convergent evolutionary pathways to spatial vision in chitons, where lineages split into two discrete trajectories that led to mutually exclusive types of visual systems. A gap in the phylomorphospace of chiton visual systems suggests that critical junctions have constrained lineages of chitons to particular evolutionary paths such that they can evolve one type of visual system but not another. Thus, no intermediate visual systems appear in the phylomorphospace. The two types of distributed visual systems of chitons rely on differing morphological innovations, like slit number in shell plates, that predate the evolution of spatial vision. Therefore, evolutionary outcomes are constrained by earlier evolutionary events. Previous studies, which

were confined to molecular experiments in laboratory environments, demonstrated that path dependence can dictate the order of adaptations and the persistence of changes across evolutionary time (2, 9, 59). Here, we have demonstrated path dependence in a naturally evolving system. Evolution is as much historical as biological, so clarifying the role of history in shaping evolutionary outcomes is critical to our understanding of the extent to which complex systems evolve in predictable ways.

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SUPPLEMENTARY MATERIALS

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 Supplementary Text
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